AHL OSMOLARITY DEPENDENCE PROTOCOL

-Bioassay with *C. violaceum* in soft agar-

This protocol was partially adapted from McClean et al. [1].

MATERIALS

- Soft agar (0.3% w/v) – 100 mL
    - Agar 0.3 g
    - NaCl 1 g
    - Tryptone 1 g
    - Yeast extract 0.5 g
    - ddH2O to 100 mL

- High Osmolarity LB Media (No NaCl, 15% sucrose) – 500 mL
    - Tryptone 5 g
    - Yeast extract 2.5 g
    - ddH2O to 250 mL
    - Autocalve
    - Filter 250 ml 30% sucrose solution into the media

- Low osmolarity LB Media (No NaCl, 0% sucrose) – 500 mL
    - Tryptone 5 g
    - Yeast extract 2.5 g
    - ddH2O to 500 mL
    - Autocalve

PROCEDURE

- Prepare overnight cultures of the construct of interest in high and low osmolarity LB media. Also prepare overnight cultures of *Chromobacterium violaceum* and a positive and negative control.

- Next day, prepare soft agar and autoclave. Let cool in a water bath at 45°C.

- Add 1 mL *C. violaceum* culture to 100 mL soft agar at 45°C. Pour the soft agar onto pre-warmed LB agar plates (5 ml/plate). Let solidify.

- Make four wells on each plate using an inverted pipette tip.

- Measure OD_{600} of the overnight cultures. If necessary, dilute so that all cultures have similar OD_{600}.

Assay with bacterial culture:

- Pipette 50 µl of low osmolarity, high osmolarity, positive control and negative control bacterial culture into separate wells.

Assay with bacterial supernatant:

- Centrifuge the bacterial cultures at 13 000 rpm for 10 minutes.
- Pipette 50 µl of low osmolarity, high osmolarity, positive control and negative control bacterial supernatant into separate wells.

- Incubate at 30°C for 16-24 hours.

DATA ANALYSIS

- Measure the radius of the purple pigmentation that has formed around the wells, using a ruler.

REFERENCES

1. McClean KH, Winson MK, Fish L, Taylor A, Chhabra SR, Camara M, et al. *Quorum sensing and Chromobacterium violaceum: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones*. Microbiology. 1997;143 (Pt 12):3703-11